INSUSCEPTIBILITY OF MONKEYS TO INOCULATION WITH BLOOD FROM MEASLES PATIENTS

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Measles, though usually a mild disease in itself, often leads to serious consequences; its involvement of the upper respiratory tract frequently facilitates the invasion of the lungs by the hæmolytic streptococcus or the pneumococcus. The resulting pneumonia has attracted considerable attention of late. Control of the hæmolytic streptococcal infections by specific vaccines or serum therapy is a difficult problem. A possible departure from this line of approach consists in the prevention of measles itself by the development of a prophylactic inoculation. The clinical features of the disease indicate the existence of an excellent theoretical basis for the development of a protective inoculation. One attack of measles confers a high degree of immunity, and the virulence of the causative organism is naturally so low that *per se* it does not produce fatal effects in man under ordinary conditions. The crucial problem is whether the virus of measles can be still further attenuated in such a manner that it would be suitable for prophylactic purposes.

On the recommendation of Colonel Frederick F. Russell the problem of prophylactic inoculation against measles is being investigated. Unfortunately, the causative organism of the disease is not known. A variety of bacteria have been cultivated from the blood and the mucous exudates of patients, but evidence of the etiologic importance of these organisms has not been produced. It has generally been considered that Hektoen,[1] by inoculations into man, has established the existence of the virus of measles in the circulating blood at least during the first day after the rash appears. Four groups of workers have reported on very slight evidence that monkeys are susceptible to the disease. These meager data constitute the essential features of the experimental work that has been conducted on measles.

Review of Literature.—In attempting the attenuation of the virus of measles, one of the first points obviously consists in determining the susceptibility of lower animals. Important symptoms have been attributed to the inoculation of the blood of measles patients into monkeys. Compiling the effects noted by various workers we have the following:

(1) Fever, (2) rash, (3) Koplik spots, (4) leucopœnia, (5) rhinitis and (6) malaise. Of these symptoms Anderson and Goldberger[2] noted especially the development of fever accompanied frequently by a rash, by coryza, rhinitis and malaise. Very significant or clearly marked results were reported for all monkeys injected with the blood taken from unquestionably early cases of measles, *i. e.*, within from 12 to 24 hours after the appearance of the rash. The very doubtful or negative results are considered as having occurred when the blood was withdrawn during the second day of the rash. Well-marked febrile reactions with an exanthem were obtained not only by the injection of serum, but also with very thoroughly washed red cells. Three species of monkeys were found susceptible to inoculation with blood, namely, *Macacus rhesus, M. cynomolgus* and *M. sinicus.*

Nicolle and Conseil[3] report the transmission of measles to a monkey (*M. sinicus*). They injected one animal with blood taken from a patient 24 hours before the appearance of the eruption; after an incubation period of nine days, a slight elevation of temperature occurred unaccompanied by any other symptoms.

Hektoen and Eggers[4] studied the leucopœnia and also the temperature reaction in *M. rhesus.* Two of three animals that were inoculated showed a transient rise in temperature; a pronounced drop in the white count was noted on the sixth day in one and on the ninth in the other, the leucopœnia persisting somewhat irregularly for several days. Differential counts showed the leucopœnia to be due principally to a drop in the polymorphonuclear cells. One animal apparently became ill on the 12th day and on the 15th a maculopapular eruption appeared and persisted for several days. No Koplik spots were seen. The development of a leucopœnia in *M. rhesus* after the inoculation of measles blood has been confirmed by Tunnicliff.[5]

Hektoen and Eggers interpret this leucopœnia as indicating a reaction to the virus of measles. Their results taken in conjunction with Anderson and Goldberger's work lead them to conclude that monkeys develop a mild kind of measles when injected with blood from patients taken soon after the rash has appeared.

Lucas and Prizer[6] selected a case of measles in a child and inoculated two monkeys with blood taken six hours before the appearance of the rash. These monkeys developed a transient erythema of the face, a leucopœnia and Koplik spots. These spots appeared on the 10th day after inoculation, but unfortunately the period of their duration was not recorded. It is not stated whether isolated spots were found or whether typical clusters occurred. No febrile reactions were obtained and the temperature of these animals was not reported. Unfortunately, these animals died about two weeks after inoculation from an intercurrent infection of unknown etiology. Several uninoculated control monkeys also died.

It is evident, then, that these studies are not very conclusive; the blood counts under these circumstances are particularly unreliable. Lucas and Prizer conclude that these monkeys were undoubtedly infected with measles.

Anderson and Goldberger, Hektoen and Eggers, Lucas and Prizer, report the successful passage of the virus of measles from monkey to monkey by the inoculation of blood. Anderson and Goldberger report six successive passages without any change in virulence. Nicolle and Conseil attempted on one occasion to subinoculate from one monkey to another, but without success.

Outline of Work.—In the following experiments, monkeys (*M. rhesus*) were inoculated from cases of measles occurring in adults 20 to 30 years of age. Blood was the only material used for inoculation, and in all cases it was withdrawn within the first 24 hours after the appearance of the rash. It was either defibrinated or collected in sodium citrate solution prepared in physiological saline. In some instances it was diluted with several parts of salt solution, according to the recommendations of Ricketts and Wilder[7] for the transmission of typhus to monkeys and also according to Hektoen and Eggers' work on measles. Observations on the inoculated animals were made each day about the middle of the forenoon, especial attention being given to the body temperature and the leucocyte count. The room temperature was also recorded, because, under normal conditions, the temperature of monkeys fluctuates somewhat. The buccal mucous membranes were examined from time to time for the appearance of Koplik spots. Excellent housing conditions were available for the animals, thereby avoiding any difficulty from the spontaneous development of rhinitis or coryza during the incubation period.

In order to test out any doubtful symptoms occurring in the inoculated monkeys, arrangements were made to inject blood from them into a susceptible human volunteer. In the selection of a human subject two points were given especial consideration, namely (1) assurance that the individual had never had a recognizable attack of measles, and (2) rejection of any individuals harboring pathogenic organisms in the respiratory passages.

It was found that very little dependence could be placed on the statement of even the educated adult that he had never had measles. Those men who could not remember an attack of the disease were asked to confer with their family; only those were accepted in whom there was no suspicion of an attack of either measles or rubella.

In addition to the routine physical examination, special care was taken to insure that the tonsils and sinuses were in good condition. In the case of enlarged tonsils, the hæmolytic streptococcus may occur in the crypts even when the surface of the gland is free from pathogenic bacteria.

Bacteriological examinations were made primarily for the hæmolytic streptococcus and pneumococcus. Examinations were also made for the meningococcus, the diphtheria and the influenza bacillus. Cultures were made over a period of from two to three weeks at intervals of from five to seven days, so that each individual was examined at least three times. As regards tuberculosis, attention was given to the history, the physical examination and the X-ray findings in the chest.

In considering the inoculation of a human subject precautions were taken against micro-organisms that might secondarily invade the blood stream of measles patients. It was necessary to avoid the possibility of carrying any such micro-organisms from the measles patients over to the monkeys and back again to a human subject. In making blood cultures in cases of measles, Tunnicliff[8] found a very considerable variety of bacteria. These secondary invaders appeared in the blood stream to an extent even greater than that occurring in scarlet fever. Therefore, we have made cultures on all specimens of blood inoculated into the monkeys.

Cultures were made aërobically in flasks of hydrocele meat infusion broth without sugar and also anaërobically according to the technique used by Plotz[9] in his work on typhus fever. For the anaërobic cultures, a few cubic centimeters of hydrocele fluid in ordinary test-tubes were inoculated with varying amounts of blood and the tubes filled with 2 per cent glucose meat infusion agar of a reaction to phenolphthalein corresponding to from 0.2 to 0.5 per cent of normal acid. Cultures were not discarded till after one month's incubation. Except for two contaminating colonies, no growth was obtained in seven cases cultured during the first day after the rash appeared. One of these patients was recultured during the second day of the rash with negative results. With a similar technique, Plotz also failed to obtain any growth from the blood of measles patients, studied as controls for his work on typhus fever.*

Results of the Inoculation of Monkeys.—Two adult monkeys were inoculated intraperitoneally, each from a different case of measles. Neither of these animals developed any symptoms that were diagnostic of the disease or that furnished any guide as to the most appropriate time for reinjection into a susceptible subject. It has been found that monkeys which fail to react to an injection of typhus blood may frequently respond to a subsequent injection; these two animals were therefore reinjected intraperitoneally with measles blood. At the same time a third normal monkey was also injected. In order to increase the opportunity for infection, these animals were injected every day for three successive days, each injection being made from fresh cases of measles.

On the first day, the normal monkey was injected into the heart and into the peritoneal cavity; all other injections of this and the other two animals were made intraperitoneally. Intracerebral inoculations were not used on account of the possible interference with the temperature reactions. There is no logical foundation for them in measles, and according to Anderson and Goldberger's results this method apparently possesses no superiority over intraperitoneal injection.

These monkeys were observed for a period of three and a half weeks after their last inoculation. No symptoms developed which differed in any essential way from changes

* Personal communication.

that were noted during control periods of observation. The temperature curves remained entirely normal. The white counts fluctuated considerably and occasionally would drop for a single day, but there was no leucopœnia that was sustained even for a short period. The temperature of Monkey I was somewhat elevated on the 15th and 16th days after the second series of inoculation, but the leucocyte count rose also at this time. In no case did we obtain the combination of a high temperature accompanied by a leucopœnia. Another monkey (II) developed a pronounced erythema with a few macules 10 days after inoculation, but this was hardly as marked as a similar rash which appeared in this animal one week before injection. It was not preceded nor accompanied by Koplik spots. The continued absence of lesions in the rather delicate mucous membrane would seem to militate against interpreting the skin eruption as due to measles. This same criticism applies to the rash without Koplik spots observed by Hektoen and Eggers.

Neither of the other two monkeys developed any lesions in the skin or mucous membranes. All three remained entirely free from conjunctivitis, rhinitis and coryza. There was no loss of appetite and no evidence of malaise.

Notwithstanding the negative results in these animals it is theoretically possible that the virus may have persisted and even multiplied without producing symptoms. Accordingly, the monkey (III) which had not previously been injected with measles was bled on the 11th day after the first of the three successive injections; after defibrination 3.5 c. c. of this blood was injected at once subcutaneously into a human volunteer. No symptoms developed. Anaërobic cultures of this blood showed no growth. Although this subject apparently never had measles, it is possible that he may have had an attack so mild as to be unrecognized yet sufficient to produce immunity. In order to obtain final proof that this specimen of blood did not contain the virus of measles it would be necessary to establish the susceptibility of this individual to measles. It is not justifiable to obtain such proof experimentally in view of the entirely negative results obtained in the monkeys.

On the basis of Hektoen's striking results it was assumed that the subcutaneous injection of a small amount of blood containing the virus of measles would reproduce the disease in man. In another series of investigations on prophylactic inoculation, a very unexpected result was obtained. One-half c. c. of serum from a fresh case of measles was inoculated subcutaneously without delay into an apparently susceptible individual; this quantity is several times larger than that used by Hektoen. This subject remained entirely free from any symptoms of the disease. This result in man casts a little additional doubt on the transmission of measles to monkeys by the inoculation of blood.

Discussion.—In the investigation of the circulating blood for the virus of measles, it would seem that trustworthy progress cannot be made by the inoculation of monkeys. Even though slight symptoms do develop in occasional individual animals it would appear that the results are too inconstant and indefinite to permit the practical use of this animal. Some more accurate means for the diagnosis of measles must be obtained before it can be clearly established that the monkey is, at times, infected by the injection of blood from early cases. It is conceivable that infection of this species with measles might be obtained in some other manner; for example, by scarifying the mucous membranes with the virus using perhaps the secretions from the respiratory tract instead of the blood of patients.

Anderson and Goldberger report that the injection into monkeys not only of bood serum, but also of very thoroughly washed corpuscles produced a well-marked reaction with an exanthem. This result suggests that the virus of measles occurs in the red cells as well as in the serum, since in the typical bacteriæmias the organisms adhering to washed corpuscles would hardly be sufficient to infect a distinctly refractory animal. However, there is good evidence that all of the diseases known to be caused by typical protozoan parasites of the red cells are transmitted by insects, whereas droplet infection constitutes the usual mode of propagation in measles. This apparently equal distribution of the virus of measles between the red cells and the serum is difficult to explain.

SUMMARY

1. Three monkeys were inoculated with the blood of measles patients taken early in the course of the disease from moderately severe cases. These animals remained entirely free from any symptoms that were either diagnostic or even suggestive of measles. Two of these animals that were injected a second time failed to develop any symptoms.

2. After an incubation period of 11 days blood was taken from one of these monkeys and injected into a human volunteer. No symptoms developed.

PROTOCOLS

The following data give the details concerning the injection of these monkeys; the record of their temperatures and white counts is given in full in order to provide the data on which we have based the interpretations given in the text. The temperature charts of the measles patients are given in order to show the stage of the febrile period at which the blood was withdrawn. This information as well as the statements concerning the time of appearance of the rash furnishes an indication of the period of the disease at which the subinoculations were made.

Monkey I was kept under observation for one week. Ten cubic centimeters of blood were taken from a case of measles (Patient A) 18 hours after the first appearance of the rash. This blood was diluted with 40 c. c. of physiological saline, then defibrinated and injected intraperitoneally. Chart I shows the temperature and white blood count of this animal. The temperature chart of the patient (Chart IV) shows that the blood was taken before the infection had begun to decline.

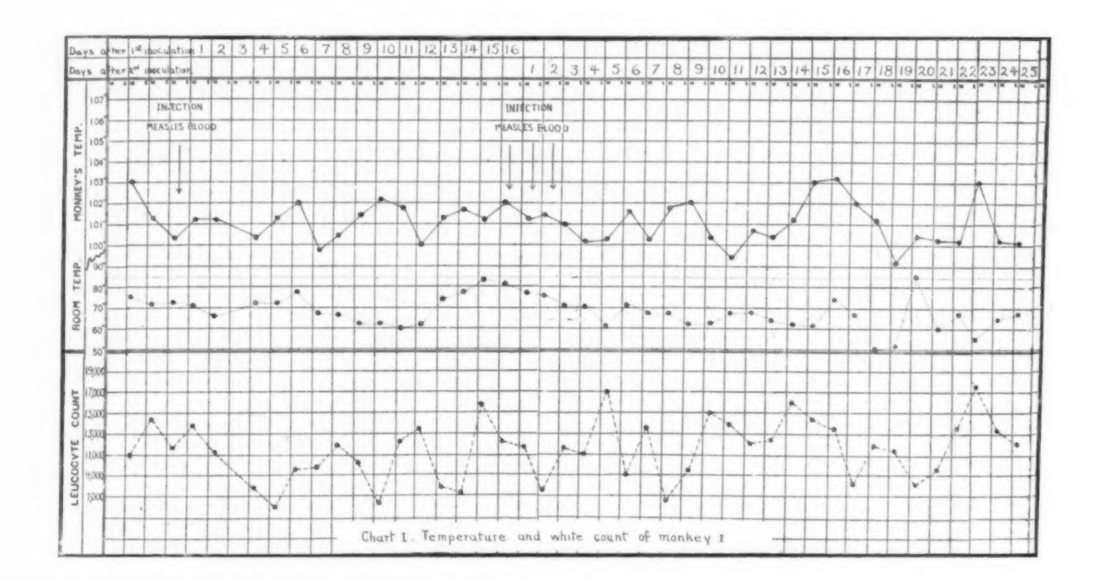


Chart I. Temperature and white count of monkey I

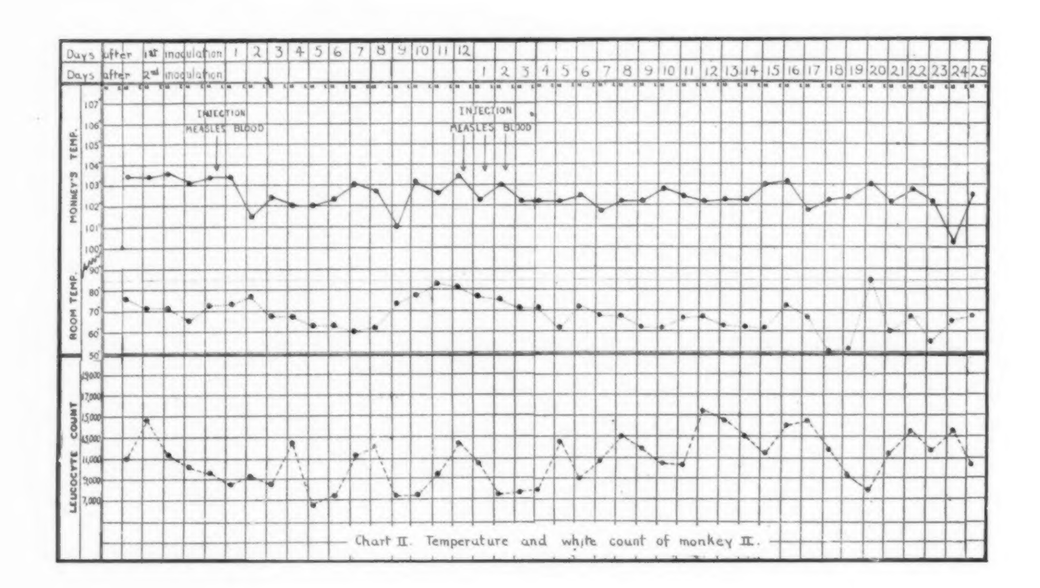


Chart II. Temperature and white count of monkey II.

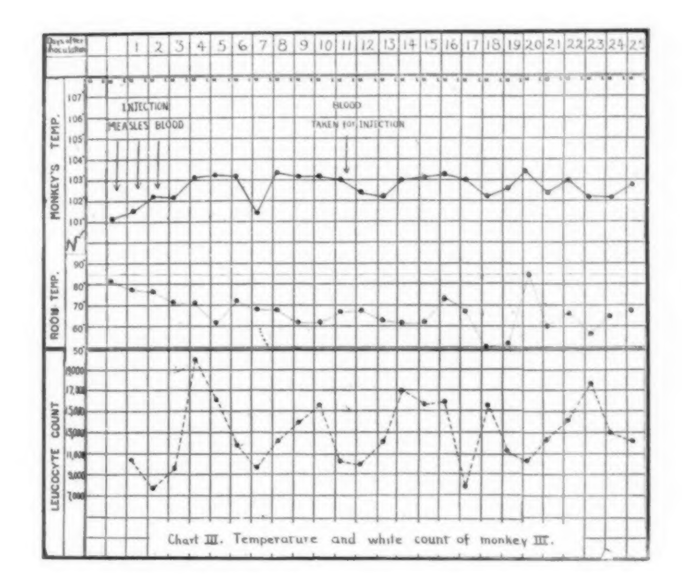


Chart III. Temperature and white count of monkey III.

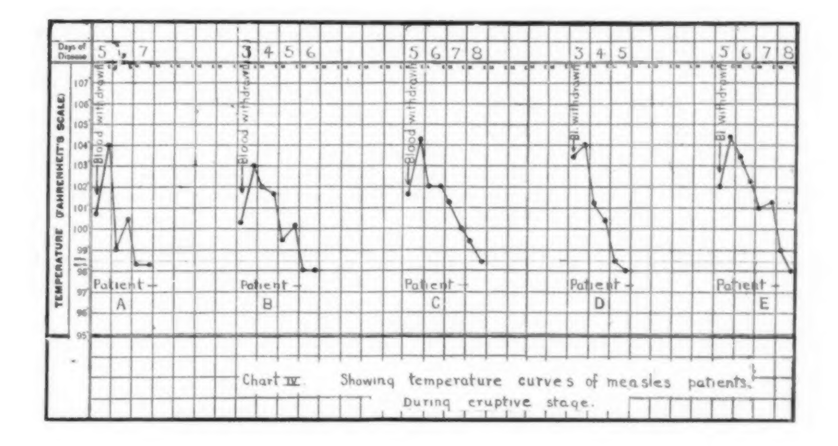


Chart IV. Showing temperature curves of measles patients. During eruptive stage.

Sixteen days after this injection, this animal was reinoculated with measles blood taken from a patient (C) within from four to six hours after the rash had appeared. The blood was mixed with an equal part of 2 per cent sodium citrate in physiological saline. Of this mixture 15 c. c. were injected intraperitoneally. On the following day, 24 hours later, another injection was made with blood taken from a patient (D) 12 hours after the appearance of the rash. This specimen of blood was diluted with an equal part of physiological saline and defibrinated; 20 c. c. of this defibrinated mixture were injected intraperitoneally. After another interval of 24 hours a final injection was made with the blood of Patient E, taken not later than 12 hours after the rash had appeared. One part of blood was mixed with two parts of physiological saline and 20 c. c. of the defibrinated mixture were injected intraperitoneally. The effect of these injections is given in Chart I. The temperature charts of these patients are also given. (Chart IV.)

Monkey II was kept under observation for 10 days. Blood for injection was taken from Patient B within from 6 to 12 hours after the first appearance of the rash. The specimen was mixed with an equal part of physiological saline and 20 c. c. of the defibrinated mixture were injected intraperitoneally. The white blood counts and temperature of this animal are given in Chart II. The temperature of the patient is recorded in Chart IV. Before injection this animal had a marked erythema over the face and eyebrows; a few macules were also present. This rash faded during the first week of the incubation period, but increased somewhat 10 days after inoculation, though its intensity was not so great as during the period before injection. Since this time it has fluctuated considerably. At present, two months after the last injection, it is more marked than at any time during the incubation periods. Twelve days after the first injection of blood this monkey received injections of measles blood on three successive days; these injections were exact duplicates of those just recorded for Monkey I, the same specimens of blood being used.

Monkey III. This animal, a young adult, was given a series of three injections simultaneously with Monkeys I and II. The same specimens of blood were employed though the details were different. The injection on the first day consisted of 8 c. c. of the mixture of citrated blood injected into the heart and 12 c. c. intraperitoneally. On the second and third days 30 c. c. of the defibrinated mixtures were given intraperitoneally. The results in this monkey are recorded in Chart III.

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THE PRODUCTION OF TETANY BY THE INTRAVENOUS INFUSION OF SODIUM BICARBONATE

REPORT OF AN ADULT CASE

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Many drugs and chemicals have been mentioned as causing tetany in adults. Following Frankl-Hochwart,[1] it has been customa to classify all these conditions in one group, as due to toxins or poisons. The clinical history of a patient with tetany which was produced, or at least precipitated, by intravenous infusions of sodium bicarbonate follows:

L. H. Medical No. 39293. Colored. Aged 22. Domestic. Admitted, March 8, 1918. Died, March 18, 1918.

The *family* and *past history* were unimportant, except that the patient remembered no previous attacks which in any way suggested tetany, and the members of her family corroborated her statement.

Present Illness.—At 7.30 a. m. on the day of admission, before she had taken any food, she swallowed two 7½-grain tablets of bichloride of mercury, dissolved in a glass of water. Shortly afterward she vomited, and within an hour commenced to vomit blood. She was admitted to the hospital about noon. At this time she was vomiting dark colored material, and was very dull and drowsy.

Physical Examination.—Nothing abnormal was made out aside from tenderness and slight muscle spasm in the upper abdomen. The throat was red, but otherwise it showed nothing. All of the deep reflexes were normally active. No stigmata of rachitis could be found. Weight, 130 pounds. Mercury was recovered from the urine, feces, and vomitus.

Blood Examination.—W. B. C., 19,000. R. B. C., 5,624,000. Hemoglobin (Sahli), 90 per cent. Differential blood count normal. The Wassermann test was negative.

Course in the Hospital.—From the time of her admission the patient passed blood in her urine and stools. The usual energetic measures were taken to secure a large fluid intake and to eliminate the mercury.

On March 9 she became totally anuric and continued so until her death. On this day the plasma bicarbonate capacity (Van Slyke) was 45 volumes per cent, and the carbon dioxide tension of the alveolar air (Marriott) was 28 mm. of mercury. She was

[1] Frankl-Hochwart: Die Tetanie der Erwachsenen, Leipzig, 1907.